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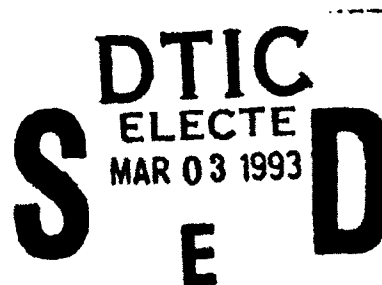
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The alphaviruses consist of a group of 26 closely related viruses. Many of these viruses can cause disease in man, characterized by encephalitis, polyarthritis, fever or rash, depending upon the virus. In the 2.5 years of research supported under this contract we have mapped antigenic epitopes in the structural glycoproteins of alphaviruses that lead to neutralization of virus infectivity upon reaction with and antibody, and have determined the sequence relationships of a number of Sindbis-like alphaviruses to one another and to other alphaviruses. We found that a domain of glycoprotein E2 of alphaviruses, between residues 170 and 220, was an important region for binding of monoclonal antibodies that neutralize virus infectivity, making it critical importance for the immune response required for protection from infection by the virus. In the determination of the relationships of alphaviruses to one another, we have determined complete or partial sequences of 8 different alphavirus RNAs. These include Ockelbo virus, a virus causing epidemic polyarthritis in northern Europe, strains of Sindbis virus from Africa, India, Australia and New Zealand and					
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Aura virus from South America. We found that the Sindbis-like viruses possess certain key features in common and are all closely related to one another. Aura virus is the first true representative of the Sindbis viruses found in the Americas and demonstrates that these viruses are global in their distribution. We have also developed improved methods for rapidly sequencing large viral RNA genomes.

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Final Report

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FOREWORD

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INTRODUCTION

The alphaviruses are a widespread group of human pathogens that are present virtually everywhere in the world (Griffin, 1986; Monath, 1988; Peters and Dalrymple, 1990). They are mosquito-borne viruses and thus are particularly prevalent in tropical areas where mosquitoes abound and problems of overwintering by the virus do not arise, but are also present in temperate areas of the world including the United States. They have the capacity to replicate in the mosquito vector as well as in human hosts or in various species of birds and mammals. Old World alphaviruses are, in general, capable of causing a painful and disabling disease in man characterized by fever, rash and arthralgia. In the cases of the Ockelbo strain of Sindbis virus and of Ross River virus, this arthralgia manifests as a polyarthrititis that may in some cases last for months or years. Many of the New World alphaviruses can cause fatal encephalitis in man. Our program attempts to understand the molecular basis of alphavirus immunogenicity and to determine the relationships of alphaviruses to one another, and has developed in collaboration with Drs. Alan Schmaljohn and Joel Dalrymple of USAMRIID.

METHODS USED

Virus Strains. Viruses used in this study were from the collection of Dr. J. M. Dalrymple of USAMRIID. Viruses were grown in BHK cells, in secondary chicken embryo fibroblast cells, or in mosquito cells, purified, and RNA prepared as described (Ou et al., 1981; Shirako et al., 1991).

cDNA Clones. cDNA clones were made in one of two ways. The first method used standard procedures in which first strand cDNA was made using oligo(dT) as primer and second strand synthesis was by the method of Gubler and Hoffman (Gubler and Hoffman, 1983; Sambrook et al., 1989). These cloning methods, as well as the methods of DNA sequencing and RNA sequencing, have been described in numerous publications from our laboratory over the years (Hahn et al., 1985; Rice et al., 1985; Rice and Strauss, 1981; Shirako et al., 1991; Strauss et al., 1984).

In a second approach, we developed methods suitable for high throughput automated DNA sequencing, in order to speed up the acquisition of sequence data. Whataroa virus was chosen as a test virus. The methods were described in detail in our annual report of 4/23/92. Briefly, first strand cDNA synthesis used random priming and second strand cDNA was synthesized by the method of Gubler and Hoffman (Gubler and Hoffman, 1983). After blunt-ending the double-stranded cDNA, the internal *EcoRI* restriction sites were methylated and the DNA was electrophoresed in an agarose gel. *EcoRI* linkers were attached to the 2-4 kb fraction and the DNA cloned in the *EcoRI* site of a suitable vector. One hundred clones that resulted from this cloning were characterized by restriction analysis and many of them were sequenced using an Applied Biosystems automated DNA sequencer.

Construction and Screening of the Bacteriophage Lambda Library. Sindbis virus strain AR339 from A. Schmaljohn at USAMRIID was grown in monolayers of primary chicken cells (Pierce et al., 1974). Virus was purified as described (Bell et al., 1978), disrupted with 0.5% SDS, and 49S genomic RNA extracted with phenol/chloroform (Hsu et al., 1973). After two ethanol precipitations, RNA was suspended in distilled water and stored at -70°C until used as a template for cDNA

synthesis. A λ gt11 expression library containing short inserts of Sindbis cDNA was constructed by a modification of the procedure of Young and Davis (Young and Davis, 1983). cDNA synthesis was randomly primed with sonicated salmon testis DNA. After flush-ending the product with the Klenow fragment of DNA polymerase I, methylation with *Eco*RI methyltransferase, and addition of *Eco*RI linkers, the modified cDNA was digested with an excess of *Eco*RI restriction enzyme. The digested DNA was fractionated on a Sephadex CL-6B column, and cDNA fragments 100-300 base pairs in size were pooled and ligated to dephosphorylated λ gt11 arms (Promega). After in vitro packaging into phage heads (Stratagene), phage plaques were grown for 6 h at 42°C. Nitrocellulose disks soaked in 10 mM isopropyl thio- β -D-galactopyranoside were then placed on top of the agar layer, and the plates were transferred to 37°C for 15 h. The filters were lifted and washed successively in 10 mM Tris-Cl pH 7.5 and 150 mM NaCl containing 5% nonfat milk. The filters were incubated overnight at 4°C with a monoclonal antibody in PBS solution containing 5% nonfat milk, washed, and the filters were then incubated at least two hours at room temperature in the presence of 125 I-conjugated protein G (0.5 μ Ci/ml in 5% nonfat milk). After washing and drying, the filters were exposed overnight at -80°C to Kodak-X-Omat film. Immunoreactive phage were picked and rescreened until a uniformly reactive population was obtained.

MAPPING OF NEUTRALIZING ANTIGENIC EPTOPES OF ALPHAVIRUSES

We have localized a site in alphavirus glycoprotein E2 that binds neutralizing antibodies. Characterization of such immunogenic domains is important in developing vaccines, because neutralizing antibodies are thought to be particularly important in protecting a vaccinee from viral infection. We

developed a novel approach in which λ gt11 expression libraries were constructed that expressed parts of the Sindbis genome, and these were screened with neutralizing monoclonal antibodies (MAbs). Many neutralizing antibodies react with discontinuous epitopes and thus will not react with a chimeric protein expressed in a λ gt11 library. However, we succeeded in identifying one MAb which bound to specific clones within the λ gt11 library (Wang and Strauss, 1991). Four λ gt11 clones were found that reacted with MAb23, and a schematic of these four clones in relation to the Sindbis virus genome is shown in Fig. 1. The four clones all contain overlapping inserts from the E2 region of the genome, and the sequence of E2 from residues 173 to 220 is present in all. This demonstrates directly that this neutralizing MAb binds to glycoprotein E2 of Sindbis virus between residues 173 and 220.

The result with MAb23 confirmed and extended results in which variants of the virus selected to be resistant to neutralizing MAbs were sequenced in order to identify the regions within the glycoproteins of the virus with which the antibodies react (Strauss et al., 1991). This is illustrated in Fig. 1 in which the sequence of E2 between residues 173 and 220 is shown, and the location of many variants that render the virus resistant to neutralization by several MAbs is indicated. It is clear that the domain between residues 170 and 220 of glycoprotein E2 of alphaviruses is particularly important for the antibody response of a host. We have estimated that 90% of the neutralizing antibodies produced by an infected mouse are directed against this E2 domain (Strauss et al., 1991)

This domain of E2 identified as being important for reactivity with neutralizing antibodies also appears to be important for virus attachment to host cell receptors. First, many neutralizing antibodies are thought to inactivate the virus by binding to the domain that interacts with the cell receptor, thus blocking virus binding to the cell. Second, antiidiotypic antibodies made to MAbs that bind

to this domain of Sindbis E2 function as antireceptor antibodies (Wang et al., 1991). Third, changes in this region of E2 alter the ability of the virus to bind to neuronal cells (Ubol and Griffin, 1991). The simplest interpretation of these results is that the E2 domain between 170 and 220 binds to a cell receptor to initiate infection.

ALPHAVIRUSES EXAMINED FOR SEQUENCE RELATIONSHIPS

We have examined 12 strains of alphaviruses for their relationships to one another. These 12 viruses are shown in Fig. 2 together with the source from which they were isolated, their year of isolation, and the location in which they were isolated. Strains to be examined were chosen in consultation with Dr. Joel Dalrymple of USAMRIID, and were chosen on the basis of geography, year of isolation, potential for human disease, and, in the case of Aura virus, as a possible parent for the emergent virus Western equine encephalitis virus.

SEQUENCE ANALYSIS OF OCKELBO VIRUS

We have determined the complete nucleotide sequence of the genome of Ockelbo virus. This virus was chosen for analysis because it causes epidemics of polyarthritis in humans, a disabling disease that can last for months. The sequence of the virus isolated in 1982 in Edsbyn, Sweden, is shown in Fig. 3. The viral genome is 11,708 nucleotides in length excluding the poly(A) tail. The genome is identical in organization to that of the Sindbis virus AR339 strain (Strauss et al., 1984) isolated in Sindbis, Egypt in 1952 (Taylor et al., 1955). There are only 672 nucleotide differences between the two viruses (5.7% divergence) that result in 97 amino acid changes (2.6) divergence. Thus more than 85% of all

Name	Strain	Source	Year	Location	Reference
Subgroup I					
Sindbis	AR339	Mosquito (<i>Culex univittatus</i>)	1952	Egypt	Taylor et al., (1955)
Sindbis	MP684	Mosquito (<i>Mansonia fuscopennata</i>)	1958	Uganda	
Sindbis	Girdwood	Human	1963	South Africa	Malherbe et al., (1963)
Sindbis	R33	Reed Warbler (<i>Acrocephalus scirpaceus</i>)	1971	Czechoslovakia	
Sindbis	1038	Turtle Dove (<i>Streptopelia turtur</i>)	1964	Israel	
Ockelbo	Edsbyn 82-5	Mosquito pool (<i>Culiseta</i> spp.)	1982	Edsbyn village, Sweden	Niklasson et al., (1984)
Ockelbo	Edsbyn 83M107	Mosquito (<i>Culiseta morsitans</i>)	1983	Edsbyn village, Sweden	
Karelian Fever	LEIV 9298	Mosquito (<i>Aedes communis</i>)	1983	Central Karelia, USSR	Lvov et al., (1984, 1988)
Subgroup II					
Sindbis	MM2215	Mosquito (<i>Culex tritaeniorhynchus</i>)	1955	Indonesia	
Sindbis	A-1036	Mite (<i>Bdellonyssus bursa</i>)	1953	India	Shah et al., (1960)
Sindbis	MRM18520	Mosquito (unidentified)	1975	Queensland, Australia	
Subgroup III					
Whataroa	M78	Mosquito pool	1962	New Zealand	
Subgroup IV					
Aura	AR10315	Mosquito (<i>Culex</i> spp.)	1959	Brazil	Causey et al. 1963

Figure 2 . Strains of Sindbis virus and related viruses used in this study.

nucleotide differences are silent, illustrating the importance of conservation of amino acid sequence.

Glycoprotein E2 is particularly important for antigenicity, as described above, and changes in E2 have been associated with changes in virulence (Lustig et al., 1988; Olmsted et al., 1986; Strauss et al., 1991; Tucker and Griffin, 1991). The differences in glycoprotein E2 between six strains of Sindbis virus are listed in Fig. 4. The residues at positions 172, 209, 212, and 216 are known to be important determinants of the antigenicity of the virus (Strauss et al., 1991), and the changes in these positions are important for the differences in the cross-reactivity of the viruses with antibodies. The residues at 55 and 172 are known to be important determinants of the neurovirulence of the virus in a mouse model (Lustig et al., 1988), and it is possible that the amino acid difference at position 55 may be important for the increased virulence of Ockelbo virus compared to the other strains of Sindbis virus in Fig. 4.

ANALYSIS OF 3' TERMINAL NONTRANSLATED SEQUENCE

To study the relationships among a number of Sindbis viruses present in nature, the sequences of the 3' nontranslated regions (NTR) were obtained for a number of strains. These sequences are shown in Fig. 5. The sequence identity throughout this region is greater than 80% for all viruses shown, and the sequence organization is identical except for a few scattered insertions and deletions. In the 3' NTR there are three repeated elements that are highly conserved (boxed in the figure). As an example of the conservation of these elements, there are 49 differences in the 3' NTRs of the Australian and AR339 strains that occur outside the repeated elements (24.1% divergence) but only 7 changes within these elements (5.8% divergence), and the overall divergence is

RESIDUE	AR339				S.A. AR86	OCKELBO
	HRSP	DG	AS	RJ		
1	S	S	S	R	S	S
3	I	T	T	T	T	T
23	V	E	E	E	E	E
29	V	V	V	V	I	I
55	Q	Q	Q	Q	Q	K
61	A	A	A	A	S	T
69	L	L	L	L	L	F
70	K	K	E	E	E	E
116	V	V	V	V	A	A
126	L	L	L	L	M	M
172	R	G	G	G	G	G
209	G	R	G	G	G	G
212	S	S	S	S	T	T
216	E	E	K	E	E	E
243	L	L	L	L	S	L
247	D	D	D	D	A	A
277	I	I	I	I	V	I
312	V	V	V	V	I	I
375	T	T	T	T	A	A
386	V	V	V	V	A	A

Figure 4. Amino acid differences in the glycoprotein E2 of various Sindbis strains. The sequence of HRSP is from Strauss et al. (1984); The sequence marked DG is the SV1A strain published in Lustig et al.(1988). AS is our unpublished sequence of the strain used by A. Schmaljohn for the isolation of antigenic escape mutants (Stec. et al., 1986); RJ is the sequence from Davis et al. (1986) of a laboratory strain from Robert Johnston. The sequence of AR86 was reported in Russell et al. (1989), and the Ockelbo sequence was presented in Figure 3.

	11290	11300	11310	11320	11330	11340
AR339 (HR)	W S W L F A L F G G A S S L L I I G L M					
Ockelbo 83	-----	-----	-----	-----	-----	-----
Ockelbo 83	-----	-----	-----	-----	-----	-----
Karelia 83	-----	-----	-----	-----	-----	-----
Girdwood 63	-----	-----	-----	-----	-----	-----
India 53	-----	AU-A	-U-G-A	-A-C-U	-G-G-A	-----
Australia 75	-C-----	-A-A-U	-G-A-U	-A-C-G	-G-G-A	-----
	11350	11360	11370	11380	11390	11400
AR339 (HR)	I F A C S M M L T S T R R Op					
Ockelbo 83	-----	-----	-----	-----	-----	-----
Ockelbo 83	-----	-----	-----	-----	-----	-----
Karelia 83	-----	-----	-----	-----	-----	-----
Girdwood 63	-----	-----	-----	???	-----	-----
India 53	-?------	GCUC-----	A-----	C-----	???	-----
Australia 75	-?------	GCUU-----	A-----	C-----	???	-----
	11410	11420	11430	11440	11450	11460
AR339 (HR)	CCGACCAGCA	AAAACUCGAUGUACUCCGAGGAACUGAUGGCAUAAUGCAUCAGGCUGGU				-----
Ockelbo 83	-----	-----	-----	-----	-----	-----
Ockelbo 83	-----	-----	-----	-----	-----	-----
Karelia 83	-----	-----	-----	-----	-----	-----
Girdwood 63	-----	-----	-----	-----	-----	-----
India 53	-----	-C-----	A-----	C-----	-----	-G-----
Australia 75	-U-----	-C-----	A-----	C-----	-----	-G-----
	11470	11480	11490	11500	11510	11520
AR339 (HR)	ACAUUAGAUC	CCCGCUUACCGCGGG	CAAUUAGCAACACUA	AAAACUCGAUGUACUCC		
Ockelbo 83	-U-----	-----U-----	-----C-----	-----C-----	-----	-----
Ockelbo 83	-U-----	-----U-----	-----C-----	-----C-----	-----	-----
Karelia 83	-U-----	-C-----U-----	-----C-----	-----C-----	-----	-----
Girdwood 63	-U-----	???	-----C-----	-----C-----	-----	-U-----
India 53	-----	???	ACCAGA-----	C-UU-----	C-G-----	C-----
Australia 75	-----	A-A-CAGA-----	UCC-----	G-G-----	-----	-U-C-----
	11530	11540	11550	11560	11570	11580
AR339 (HR)	GAGGAAGCGCAGUGCAUAAUGC		UGCGCAGUGUUGCCACAUA	ACCACUAUAUUA	ACCAUUU	-----
Ockelbo 83	-----	-----	-----	U-U-----	-----	-----
Ockelbo 83	-----	-----	-----	U-U-----	-----	-----
Karelia 83	-----	-----	-----	U-U-----	-----	-----
Girdwood 63	-----	-----	-----	A-U-----	-----	-----
India 53	-----	-U-----	-C-----	??-UU-U-----	UU-----	UU-A-----
Australia 75	-----	-U-----	-A-----	C-C-U-----	UU-U-----	UU-A-----
	11590	11600	11610	11620	11630	11640
AR339 (HR)	AUCUAGCGGACGCCA	AAAACUCAUUGUAUUUCUGAGGAAGCGUGGUGCAUAAUGCCACCGC				-----
Ockelbo 83	-U-----	G-----	-----	A-----	-----	U-----
Ockelbo 83	-U-----	G-----	-----	A-----	C-----	U-----
Karelia 83	-U-----	G-----	-----	A-C-----	-----	U-----
Girdwood 63	-U-----	-----	-----	A-----	-----	U-----
India 53	-AG-UA-----	A-----	-----	-----	-----	-----
Australia 75	-CAG-UA-----	A-----	-----	-----	-----	-----
	11650	11660	11670	11680	11690	11700
AR339 (HR)	AGCGUCUGCAUAAC	UUUUAUUA	UUUCUUUA	UUAUCAACAAAAUUUUGUUUUUAACA	UUUC	-poly (A)
Ockelbo 83	-----	-C--UU-----	-----	-----	-----	-----
Ockelbo 83	-----	-----U-----	-----	-----	-----	-----
Karelia 83	-----	-U-----U-----	-----	-----	-----	-----
Girdwood 63	-----	-----U-----	-----	-----	-----	-----
India 53	-U-----	A-----CAAC-----	-----	U-UG-UU-----	U-----	NNNN--
Australia 75	-U-----	AA-----UCAA-----	-----	U-U-----U-----	-----	NNNN--

Figure 5. Sequence of the 3' termini of several Sindbis viruses. The sequences of Ockelbo 83M107, Karelian fever, and South African Sindbis (Girdwood) were determined from cloned cDNA. Those of the Indian A1036 and Australian 18520 isolates were determined directly from RNA by dideoxy sequencing using reverse transcriptase and a T12GA primer. The Ockelbo 82 sequence is from Fig. 3 and that of AR339 (HRSP) is from Strauss et al. (1984). Three repeated sequence elements of 40 nucleotides are boxed. The translated sequence is for AR339 (HRSP) and any amino acid that differs in the other viruses is boxed. This figure is from Shirako et al. (1991).

18.1%. From such analysis, we propose that these repeated and conserved elements play an important role in viral RNA replication, and this role is probably more important in mosquito cells than in vertebrate cells (Kuhn et al., 1990).

The relationships among these viruses is illustrated in Fig. 6. Three points are obvious from this diagram. One is that the Sindbis strains analyzed can be divided into a European-African group and an Asian-Australian group. The second point is that Ockelbo virus and Karelian fever virus are virtually identical. The third point is that Ockelbo virus is more closely related to the South African strain of Sindbis virus isolated in 1963 (and which is also capable of causing human illness) than it is to the Egyptian strain isolated in 1952. We conclude from this last point that Ockelbo virus was probably introduced into Northern Sweden from South Africa in the 1960s, from where it spread into Finland (where it causes the disease called Pogosta) and the Karelian region of Russia.

SEQUENCE STUDIES OF AURA RNA

We have obtained the sequence of essentially all of the genome of Aura virus and are currently assembling this sequence. We were particularly interested in this virus because we have previously shown that Western equine encephalitis virus (WEE), previously thought to be closely related to Sindbis virus, is in fact a recombinant virus in which most of the genome was derived from Eastern equine encephalitis virus and only the surface glycoproteins were derived from a Sindbis-like virus (Hahn et al., 1988). Thus the question arose as to whether there is a virus found in the Americas that is closely related to Sindbis and that could have served as the second parent of WEE. The question is of particular interest because WEE emerged from a recombination event.

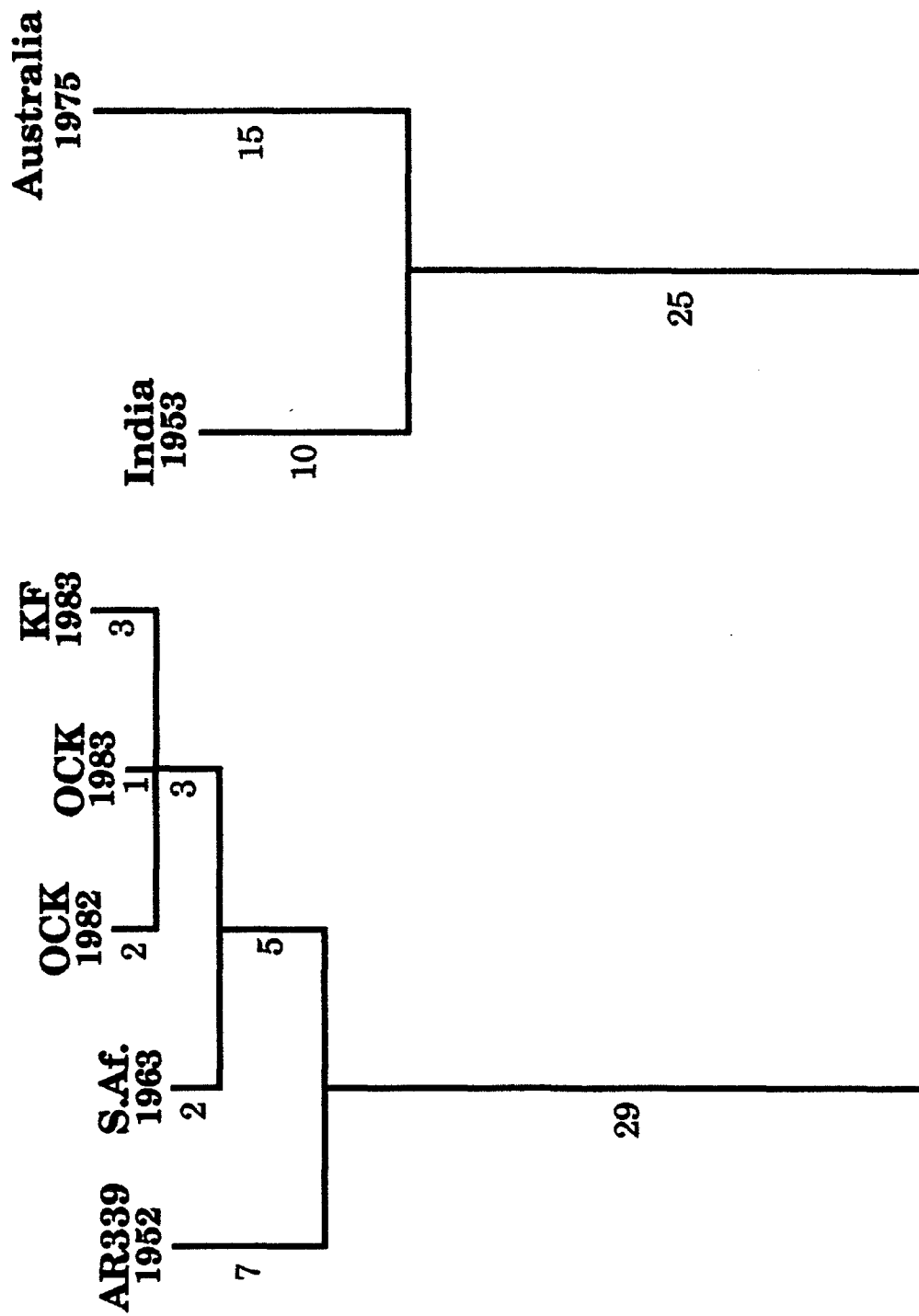


Figure 6 . Evolutionary tree of strains of Sindbis virus. The vertical distances indicate the number of nucleotide differences between any two strains in the 3'terminal 420 nucleotides. The horizontal distances are arbitrary. The tree was constructed from a difference matrix by iteration to give the best possible representation with the minimum number of branch points. Nucleotide differences between any two strains can be calculated by summing the numbers on the vertical branches connecting the two strains to be compared.

The sequence of about 5000 nucleotides of Aura RNA in the nonstructural protein coding region is shown in Fig. 7. This sequence begins in the 5' NTR and continues through nsP1, nsP2, and part of nsP3. From this sequence, it is clear that Aura virus is closely related to Sindbis virus. Comparison of the amino acid sequences of Sindbis virus and of Aura virus in the region represented by the Aura sequence in Fig. 7 shows that the two sequences are 80% identical, illustrating that Aura is in fact a Sindbis-like virus. We also found that the 3' NTR of Aura RNA is Sindbis-like. As described above, Sindbis-like viruses contain three copies of a conserved sequence element that we postulate is important for RNA replication. Although other alphaviruses often contain repeated sequence elements, these elements are completely different in sequence from the Sindbis sequence. Furthermore, WEE lacks the characteristic Sindbis 3' NTR, and contains instead a chimeric 3' NTR. Thus Aura virus represents the first known example of a true Sindbis-like virus in the Americas

Aura virus is widely distributed in South America, having been isolated in Brazil and in Northern Argentina. Analysis of the data is not yet complete, but it is possible that Aura virus represents the ancestral Sindbis-like virus, and that it was transmitted to the Old World to serve as the founder of the Sindbis viruses in the Old World, as we previously postulated (Levinson et al., 1990). Aura virus may have served as one of the parents of WEE, contributing its glycoproteins to this recombinant virus (Hahn et al., 1988).

1	ACT AGT ACT TGT ACT ACA GAA TTA ACT GCC GTG TGC CGC CCG CTA AAC TAG CCC CAA TCA
61	TCG AAA ATG GAG AAA CCG ACA GTG CAC GTT GAC GTA GAC CCC CAA AGT CCG TTT GTG CTA met glu lys pro thr val his val asp val asp pro gln ser pro phe val leu
121/19	CAA CTG CAG AAG AGT TTC CCA CAA TTC GAG ATT GTG GCT CAG CAG GTC ACT CCG AAT GAC gln leu gln lys ser phe pro gln phe glu ile val ala gln gln val thr pro asn asp
181/39	CAT GCT AAT GCC AGA GCT TTT TCG CAT CTG GCT AGT AAA CTG ATC GAA CAT GAG ATC CCC his ala asn ala arg ala phe ser his leu ala ser lys leu ile glu his glu ile pro
241/59	ACC TCA GTT ACG ATC TTG GAC ATA GGA AGC GCA CCA GCT CGT AGA ATG TAT TCC GAG CAT thr ser val thr ile leu asp ile gly ser ala pro ala arg arg met tyr ser glu his
301/79	AAG TAT CAC TGT GTG TGC CCC ATG CGT AGT CCT GAA GAC CCG GAC CGT CTT ATG AAT TAC lys tyr his cys val cys pro met arg ser pro glu asp pro asp arg leu met asn tyr
361/99	GCA TCC CGA CTC GCA GAC AAA GCA GGG GAA ATT ACC AAC AAG AGG CTG CAT GAT AAA CTT ala ser arg leu ala asp lys ala gly glu ile thr asn lys arg leu his asp lys leu
421/119	GCA GAC CTC AAG TCG GTC CTC GAG TCG CCG GAT GCT GAA ACT GGT ACC ATT TGT TTC CAC ala asp leu lys ser val leu glu ser pro asp ala glu thr gly thr ile cys phe his
481/139	AAT GAC GTA ATA TGC CGT ACG ACA GCG GAG GTA TCA GTT ATG CAA AAT GTG TAT ATC AAT asn asp val ile cys arg thr thr ala glu val ser val met gln asn val tyr ile asn
541/159	GCA CCT TCG ACC ATT TAC CAT CAG GCC CTA AAG GGA GTC AGA AAA CTG TAT TGG ATC GGG ala pro ser thr ile tyr his gln ala leu lys gly val arg lys leu tyr trp ile gly
601/179	TTC GAT ACA ACG CAG TTT ATG TTC TCC TCG ATG GCA GGG TCG TAT CCG TCC TAC AAT ACT phe asp thr thr gln phe met phe ser ser met ala gly ser tyr pro ser tyr asn thr
661/199	AAT TGG GCC GAT GAA AGG GTG CTG GAA GCG CGT AAT ATA GGC CTA TGT AGC ACG AAG CTG asn trp ala asp glu arg val leu glu ala arg asn ile gly leu cys ser thr lys leu
721/219	AGA GAG GGT ACG ATG GGC AAA CTG TCT ACC TTC CGG AAA AAG GCC TTG AAA CCT GGA ACT arg glu gly thr met gly lys leu ser thr phe arg lys lys ala leu lys pro gly thr
781/239	AAC GTG TAC TTC TCT GTC GGT TCG ACA CTC TAC CCT GAG AAT AGA GCG GAC CTG CAG AGT asn val tyr phe ser val gly ser thr leu tyr pro glu asn arg ala asp leu gln ser
841/259	TGG CAC CTA CCA TCT GTG TTC CAC TTG AAA GGT AAA CAA TCC TTT ACG TGC CGC TGT GAT trp his leu pro ser val phe his leu lys gly lys gln ser phe thr cys arg cys asp
901/279	ACG GCG GTT AAC TGC GAA GGA TAC GTA GTC AAG AAG ATC ACC ATC AGC CCC GGG ATC ACG thr ala val asn cys glu gly tyr val val lys lys ile thr ile ser pro gly ile thr
961/299	GGG CGT GTC AAT CGG TAC ACT GTG ACT AAC AAC AGC GAG GGA TTC TTG CTG TGT AAG ATC gly arg val asn arg tyr thr val thr asn asn ser glu gly phe leu leu cys lys ile
1021/319	ACA GAT ACG GTC AAA GGG GAG CGT GTA TCG TTC CCT GTC TGT ACG TAT ATT CCA CCT TCA thr asp thr val lys gly glu arg val ser phe pro val cys thr tyr ile pro pro ser
1081/339	ATC TGT GAC CAA ATG ACA GGT ATA TTG GCC ACT GAT ATC CAA CCC GAA GAC GCG CAA AAG ile cys asp gln met thr gly ile leu ala thr asp ile gln pro glu asp ala gln lys

Figure 7a. See legend on last page of this sequence

1141/359 TTG CTG GTA GGA CTG AAC CAA CGC ATA GTC GTG AAC GGA AAA ACT AAT AGA AAC ACC AAC
leu leu val gly leu asn gln arg ile val val asn gly lys thr asn arg asn thr asn

1201/379 ACG ATG CAG AAC TAT CTC CTG CCC GCG GTG GCT ACA GGT CTG AGT AAA TGG GCC AAA GAA
thr met gln asn tyr leu leu pro ala val ala thr gly leu ser lys trp ala lys glu

1261/399 AGA AAG GCA GAC TGC AGT GAC GAG AAA CCA TTG AAT GTG AGA GAA CGC AAA CTA GCT TTC
arg lys ala asp cys ser asp glu lys pro leu asn val arg glu arg lys leu ala phe

1321/419 GGT TGC CTA TGG GCT TTC AAG ACC AAG AAG ATC CAT TCT TTT TAC CGC CCG CCA GGC ACG
gly cys leu trp ala phe lys thr lys lys ile his ser phe tyr arg pro pro gly thr

1381/439 CAG ACT ATA GTA AAA GTC GCA GCG GAA TTC AGT GCG TTC CCT ATG TCC TCG GTG TGG ACT
gln thr ile val lys val ala ala glu phe ser ala phe pro met ser ser val trp thr

1441/459 ACG TCA CTG CCA ATG TCA CTG AGA CAG AAA GTT AAA CTG CTT CTT GTA AAG AAA ACC AAT
thr ser leu pro met ser leu arg gln lys val lys leu leu leu val lys lys thr asn

1501/479 AAA CCG GTA GTC ACT ATT ACT GAC ACT GCG GTA AAA AAC GCA CAA GAG GCA TAT AAC GAA
lys pro val val thr ile thr asp thr ala val lys asn ala gln glu ala tyr asn glu

1561/499 GCC GTC GAG ACA GCA GAA GCG GAG GAG AAA GCG AAG GCC TTA CCT CCG CTG AAG CCG ACG
ala val glu thr ala glu ala glu glu lys ala lys ala leu pro pro leu lys pro thr

1621/519 GCA CCC CCT GTA GCG GAG GAC GTC AAA TGC GAG GTC ACC GAC CTG GTA GAC GAT GCG GGA
ala pro pro val ala glu asp val lys cys glu val thr asp leu val asp asp ala gly

1681/539 GCG GCC CTG GTC GAG ACG CCC CGG GGA AAG ATA AAA ATT ATC CCA CAG GAA GGG GAC GTG
ala ala leu val glu thr pro arg gly lys ile lys ile ile pro gln glu gly asp val

1741/559 CGT ATT GGT TCC TAC ACA GTC ATT TCT CCA GCG GCA GTC CTT AGA AAT CAA CAA CTG GAG
arg ile gly ser tyr thr val ile ser pro ala ala val leu arg asn gln gln leu glu

1801/579 CCA ATC CAC GAG TTA GCA GAG CAG GTG AAA ATT ATC ACG CAC GGT GGC CGA ACA GGC AGG
pro ile his glu leu ala glu gln val lys ile ile thr his gly gly arg thr gly arg

1861/599 TAT TCC GTC GAA CCT TAC GAT GCT AAG GTT CTC CTG CCA ACA GGA TGC CCC ATG TCC TGG
tyr ser val glu pro tyr asp ala lys val leu leu pro thr gly cys pro met ser trp

1921/619 CAA CAT TTC GCG GCC TTG AGC GAA AGC GCT ACG TTA GTC TAC AAT GAG AGA GAG TTC CTG
gln his phe ala ala leu ser glu ser ala thr leu val tyr asn glu arg glu phe leu

1981/639 AAC CGG AAA CTC CAT CAC ATC GCT ACG AAG GGT GCG GCA AAA AAC ACT GAG GAA GAA CAA
asn arg lys leu his his ile ala thr lys gly ala ala lys asn thr glu glu glu gln

2041/659 TAC AAA GTA TGC AAA GCT AAA GAC ACG GAT CAT GAG TAC GTA TAC GAC GTA GAT GCC AGA
tyr lys val cys lys ala lys asp thr asp his glu tyr val tyr asp val asp ala arg

2101/679 AAA TGC GTA AAA AGA GAG CAT GCA CAA GGG CTA GTA CTA GTT GGG GAA CTA ACT AAT CCG
lys cys val lys arg glu his ala gln gly leu val leu val gly glu leu thr asn pro

2161/699 CCT TAC CAC GAG CTG GCA TAC GAA GGA TTA CGT ACA CGA CCC GCT GCC CCT TAC CAT ATC
pro tyr his glu leu ala tyr glu gly leu arg thr arg pro ala ala pro tyr his ile

Figure 7b. See legend on last page of this sequence

2221/719 GAA ACA CTG GGG GTC ATT GGA ACA CCG GGG TCA GGT AAG TCG GCC ATC ATA AAA TCT ACG
glu thr leu gly val ile gly thr pro gly ser gly lys ser ala ile ile lys ser thr

2281/739 GTA ACA CTA AAA GAC CTC GTA ACT AGC GGT AAG AAA GAA AAT TGC AAA GAA ATA GAG AAT
val thr leu lys asp leu val thr ser gly lys lys glu asn cys lys glu ile glu asn

2341/759 GAC GTC CAG AAA ATG CGG GGA ATG ACT ATA GCT ACG AGA ACG GTA GAC TCG GTA CTT CTT
asp val gln lys met arg gly met thr ile ala thr arg thr val asp ser val leu leu

2401/779 AAT GGA TGG AAG AAA GCA GTA GAC GTC CTA TAT GTG GAT GAA GCG TTT GCA TGT CAT GCA
asn gly trp lys lys ala val asp val leu tyr val asp glu ala phe ala cys his ala

2461/799 GGC ACC TTA ATG GCA TTG ATT GCC ATT GTC AAA CCG AGA CGT AAA GTA GTA CTG TGC GGC
gly thr leu met ala leu ile ala ile val lys pro arg arg lys val val leu cys gly

2521/819 GAC CCG AAG CAG TGG CCC TTC TTT AAT TTA ATG CAA CTG AAG GTA AAC TTC AAC AAC CCC
asp pro lys gln trp pro phe phe asn leu met gln leu lys val asn phe asn asn pro

2581/839 GAG CGA GAC CTG TGT ACT TCC ACC CAT TAT AAA TAT ATC TCT CGC AGG TGC ACC CAA CCT
glu arg asp leu cys thr ser thr his tyr lys tyr ile ser arg arg cys thr gln pro

2641/859 GTT ACA GCC ATA GTG TCT ACA TTA CAC TAT GAC GGA AAG ATG AGG ACT ACG AAT CCC TGC
val thr ala ile val ser thr leu his tyr asp gly lys met arg thr thr asn pro cys

2701/879 AAA AGG GCT ATC GAA ATA GAC GTA AAC GGA TCG ACT AAG CCC AAG AAA GGA GAC ATA GTG
lys arg ala ile glu ile asp val asn gly ser thr lys pro lys lys gly asp ile val

2761/899 TTG ACG TGT TTC CGT GGG TGG GTT AAG CAG GGG CAA ATC GAT TAC CCC GGA CCC GGA GGT
leu thr cys phe arg gly trp val lys gln gly gln ile asp tyr pro gly pro gly gly

2821/919 CAT GAC CGT GCA GCT TCT CAA GGG CTA ACC AGA AGG GGC GTT TAT GCG GTC AGA CAG AAA
his asp arg ala ala ser gln gly leu thr arg arg gly val tyr ala val arg gln lys

2881/939 GTA AAT GAA AAC CCA CTA TAT GCA GAG AAG TCA GAA CAC GTT AAC GTG TTA CTT ACT AGG
val asn glu asn pro leu tyr ala glu lys ser glu his val asn val leu leu thr arg

2941/959 ACG GAA GAT CGC ATA GTG TGG AAG ACA CTG CAA GGG GAT CCT TGG ATT AAG TAC CTC ACT
thr glu asp arg ile val trp lys thr leu gln gly asp pro trp ile lys tyr leu thr

3001/979 AAC GTT CCA AAA GGG AAC TTT ACA GCC ACT TTA GAA GAA TGG CAG GCG GAA CAC GAG GAC
asn val pro lys gly asn phe thr ala thr leu glu glu trp gln ala glu his glu asp

3061/999 ATT ATG AAG GCC ATT AAT TCT ACA TCC ACA GTA TCT GAC CCT TTC GCC AGC AAA GTG AAT
ile met lys ala ile asn ser thr ser thr val ser asp pro phe ala ser lys val asn

3121/1019 ACA TGC TGG GCT AAA GCT ATT ATA CCC ATC CTA AGA ACG GCA GGG ATA GAA CTT ACA TTC
thr cys trp ala lys ala ile ile pro ile leu arg thr ala gly ile glu leu thr phe

3181/1039 GAG CAG TGG GAA GAT CTA TTC CCG CAA TTT CGT AAT GAC CAA CCT TAC TCC GTG ATG TAT
glu gln trp glu asp leu phe pro gln phe arg asn asp gln pro tyr ser val met tyr

3241/1059 GCC CTA GAT GTG ATA TGT ACC AAG ATG TTC GGC ATG GAT CTG AGC AGT GGG ATC TTC TCT
ala leu asp val ile cys thr lys met phe gly met asp leu ser ser gly ile phe ser

3301/1079 CGT CCT GAG ATA CCT CTA ACG TTC CAT CCC GCG GAC GTC GGC CGA GTG AGA GCT CAC TGG
arg pro glu ile pro leu thr phe his pro ala asp val gly arg val arg ala his trp

Figure 7c. See legend on last page of this sequence

3361/1099 GAT AAC TCC CCA GGA GGG CAG AAG TTT GGG TAT AAC AAG GCG GTA ATC CCA ACT TGC AAG
asp asn ser pro gly gly gln lys phe gly tyr asn lys ala val ile pro thr cys lys

3421/1119 AAA TAC CCA GTG TAC TTA AGA GCA GGA AAA GGG GAC CAA ATA CTC CCC ATA TAT GGC AGA
lys tyr pro val tyr leu arg ala gly lys gly asp gln ile leu pro ile tyr gly arg

3481/1139 GTT TCA GTC CCA TCG GCA CGG AAC AAT TTA GTT CCC TTA AAC AGA AAT CTA CCA CAC TCG
val ser val pro ser ala arg asn asn leu val pro leu asn arg asn leu pro his ser

3541/1159 CTA ACT GCA AGC CTG CAG AAA AAA GAA GCA GCT CCC TTG CAC AAG TTC CTT AAC CAA CTA
leu thr ala ser leu gln lys lys glu ala ala pro leu his lys phe leu asn gln leu

3601/1179 CCA GGA CAC AGT ATG CTG CTG GTC TCT AAG GAA ACA TGC TAT TGC GTG TCC AAG CGA ATC
pro gly his ser met leu leu val ser lys glu thr cys tyr cys val ser lys arg ile

3661/1199 ACA TGG GTC GCT CCG CTG GGA GTC AGA GGA GCT GAC CAC AAC CAT GAC CTG CAT TTC GGG
thr trp val ala pro leu gly val arg gly ala asp his asn his asp leu his phe gly

3721/1219 TTC CCA CCA CTG TCC AGA TAC GAC CTT GTG GTG GTT AAT ATG GGA CAA CCG TAC AGG TTC
phe pro pro leu ser arg tyr asp leu val val val val asn met gly gln pro tyr arg phe

3781/1239 CAT CAC TAC CAG CAG TGC GAG GAG CAT GCC GGC CTC ATG AGG ACG TTG GCC CGG TCA GCA
his his tyr gln gln cys glu glu his ala gly leu met arg thr leu ala arg ser ala

3841/1259 CTC AAC TGC CTA AAA CCA GGA GGA ACA TTA GCC CTG AAA GCA TAT GGT TTC GCC GAC TCC
leu asn cys leu lys pro gly gly thr leu ala leu lys ala tyr gly phe ala asp ser

3901/1279 AAT AGT GAG GAC GTT GTT CTG TCT TTA GCG AGG AAA TTC GTG CGG GCA TCC GCA GTG AGA
asn ser glu asp val val leu ser leu ala arg lys phe val arg ala ser ala val arg

3961/1299 CCA TCG TGT ACA CAG TTT AAC ACA GAG ATG TTC TTT GTA TTT AGG CAG CTG GAC AAC GAT
pro ser cys thr gln phe asn thr glu met phe phe val phe arg gln leu asp asn asp

4021/1319 CGT GAG CGC CAA TTC ACT CAG CAT CAC TTG AAT TTA GCA GTA TCC AAT ATA TTC GAC AAT
arg glu arg gln phe thr gln his his leu asn leu ala val ser asn ile phe asp asn

4081/1339 TAT AAA GAC GGA TCC GGA GCA GCT CCT TCT TAT CGC GTT AAG AGA ATG AAT ATC GCA GAC
tyr lys asp gly ser gly ala ala pro ser tyr arg val lys arg met asn ile ala asp

4141/1359 TGC ACA GAA GAA GCA GTG GTG AAC GCA GCT AAC GCG CGG GGA AAA CCT GGG GAC GGA GTA
cys thr glu glu ala val val asn ala ala asn ala arg gly lys pro gly asp gly val

4201/1379 TGC AGA GCT ATC TTC AAA AAG TGG CCG AAG TCA TTT GAG AAC GCT ACC ACT GAA GTG GAA
cys arg ala ile phe lys lys trp pro lys ser phe glu asn ala thr thr glu val glu

4261/1399 ACC GCG GTC ATG AAA CCA TGC CAC AAC AAG GTT GTT ATA CAT GCA GTG GGT CCT GAT TTT
thr ala val met lys pro cys his asn lys val val ile his ala val gly pro asp phe

4321/1419 AGA AAG TAC ACG TTG GAG GAA GCG ACG AAG CTA CTG CAG AAC GCA TAC CAT GAT GTG GCA
arg lys tyr thr leu glu glu ala thr lys leu leu gln asn ala tyr his asp val ala

4381/1439 AAG ATA GTG AAC GAG AAA GGC ATC TCC TCG GTA GCT ATA CCG CTG CTC TCA ACA GGT ATC
lys ile val asn glu lys gly ile ser ser val ala ile pro leu leu ser thr gly ile

4441/1459 TAT GCT GCC GGA GCT GAT CGC CTG GAT CTC TCG CTG AGA TGT CTT TTC ACC GCG CTG GAT
tyr ala ala gly ala asp arg leu asp leu ser leu arg cys leu phe thr ala leu asp

Figure 7d. See legend on last page of this sequence

4501/1479 CGT ACG CAT GCG GAT GTC ACA ATA TAT TGC CTA GAT AAG AAG TGG GAG CAA CGC ATA GCA
 arg thr asp ala asp val thr ile tyr cys leu asp lys lys trp glu gln arg ile ala
 4561/1499 GAT GCT ATT AGG ATG CGA GAA CAA GTA ACT GAA TTA AAA GAT CCG GAC ATA GAG ATA GAT
 asp ala ile arg met arg glu gln val thr glu leu lys asp pro asp ile glu ile asp
 4621/1519 GAA GGA TTA ACC CGG GTA CAC CCA GAT AGC TGC CTC AAG GAT CAC ATA GGC TAC AGT ACC
 glu gly leu thr arg val his pro asp ser cys leu lys asp his ile gly tyr ser thr
 4681/1539 CAG TAT GGG AAA TTG TAC TCA TAC TTT GAA GGT ACT AAA TTC CAC CAA ACC GCA AAA GAC
 gln tyr gly lys leu tyr ser tyr phe glu gly thr ly phe his gln thr ala lys asp
 4741/1559 ATA GCC GAG ATT CGT GCG CTG TTT CCT GAT GTA CAA GCC GCT AAC GAA CAA ATC TGC CTG
 ile ala glu ile arg ala leu phe pro asp val gln ala ala asn glu gln ile cys leu
 4801/1579 TAC ACT TTA GGC GAA CCG ATG GAG TCC ATA CGC GAA AAG TGC CCA GTC GAA GAC TCC CCG
 tyr thr leu gly glu pro met glu ser ile arg glu lys cys pro val glu asp ser pro
 4861/1599 GCA TCA GCA CCT CCT AAG ACA ATA CCT TGC CTA TGT ATG TAT GCT ATG ACA GCC GAA CGT
 ala ser ala pro pro lys thr ile pro cys leu cys met tyr ala met thr ala glu arg
 4921/1619 ATT TGC CGC GTA CGC AGT AAC TCC GTA ACG AAC ATA ACG GTG TGC TCA TCC TTT CCG TTA
 ile cys arg val arg ser asn ser val thr asn ile thr val cys ser ser phe pro leu
 4981/1639 CCC AAG TAC CGA ATA AAG AAC GTA CAA AAG ATA CAA TGC ACG AAA GTG
 pro lys tyr arg ile lys asn val gln lys ile gln cys thr lys val

Figure 7e. Translated sequence of Aura virus. This sequence starts near the 5' terminus of the genome, although the exact 5' end is not known. The translated sequence shown encompasses nsP1, nsP2, and the N-terminal (conserved) region of nsP3. Nucleotides are numbered from the beginning of the sequence; amino acids are numbered from the beginning of the open reading frame.

SEQUENCE ANALYSIS OF WHATAROA VIRUS.

We have obtained most of the sequence of Whataroa virus RNA, 11.7 kb in length. This sequence is being assembled to give the complete sequence of this virus RNA. We were interested in this virus because it represents a geographically isolated Sindbis-like virus, being found in New Zealand and presumably transferred there by migratory birds.

The sequences of a stretch of the nonstructural protein coding region of the Whataroa genome is shown in Figs 8. The sequence begins near the beginning of the nsP2 gene and continues through to the end of the nsP2 region of the virus genome, a stretch of about 2000 nucleotides. From the analysis of this sequence, Whataroa virus can clearly be considered to be a strain of Sindbis virus that has spread to New Zealand. The amino acid sequence deduced from the nucleotide sequence in Fig. 8 is compared to that of the AR339 strain of Sindbis virus, isolated from Egypt in 1952, in Fig. 9. These amino acid sequences are 84% identical. Furthermore, we found that Whataroa virus RNA has the characteristic 3' NTR of the Sindbis viruses.

SEQUENCE ANALYSIS OF OTHER ALPHAVIRUSES

We have obtained the nucleotide sequence encoding the nsP3 and nsP4 genes of several other alphaviruses, in order to examine the relationships of viruses isolated from Australia, India, and South Africa to other alphaviruses. Sequences of this region for Sindbis virus isolated from India in 1953 is shown in Fig. 10, that for a Sindbis virus isolated in Australia in 1975 is shown in Fig. 11, and that for a Sindbis virus isolated from South Africa in 1963 is shown in Fig. 12. The South African isolated came from a human patient exhibiting symptoms of

1	F I N R K L Y H I A V H G P A K N T E E	20
1	TTCATTAACAGGAAATTGTACCACATTGCAGTTCATGGTCCC CGGAAGAATACTGAGGAA	60
21	E Q Y K A M R A E A A D T E Y V F D V D	40
61	GAGCAGTATAAAGCTATGAGAGCAGAAGCGGCGGACACCGAATATGTCTTCGATGTCGAC	120
41	K K K C V K R E E A S G L V L V G E L T	60
121	AAGAAGAAGTGC GTTAAGAGAGAAGAAGCATCGGGTCTTGTGTTAGTAGGCGAACTTACC	180
61	N P P Y H E M A L E G L K T R P A V P Y	80
181	AACCCGCCATACCATGAAATGGCGCTGGAAGGGCTGAAGACCCGTCCTGCAGTACCTTAT	240
81	K V E T I G V I G T P G S G K S A I I K	100
241	AAAGTTGAAACAATCGGAGTCATCGGCACACCGGGATCCGGAAAATCCGCAATCATTA	300
101	N I V T T R D L V T S G K K E N C R E I	120
301	AACATCGTCACTACCAGGGATCTTGTGACCAGCGGAAAGAAAGAAACTGCCGGGAAATA	360
121	E A D V L K H R K M Q I V S K T V D S V	140
361	GAAGCTGACGTCCTCAAACACCGAAAAATGCAAATCGTTTCAAAGACGGTCGACTCCGTT	420
141	L L N G C H K S V D I L Y V D E A Y A C	160
421	TTGCTTAATGGTTGCCACAAGTCAGTCGACATCCTGTATGTCGACGAAGCTTACGCGTGC	480
161	H A G T L L A L I A I V R P R N K V V L	180
481	CACGCTGGCACCCTATTGGCCTTAATCGCCATAGTCCGACCTAGAAATAAAGTGGTCCTA	540
181	C G D P K Q C G F F N M M Q L K V H F N	200
541	TGTGGCGACCCAAAACAGTGTGGTTTCTTCAACATGATGCAGCTGAAGGTCCACTTTAAC	600
201	D P E R D I C T K T F Y K Y I S R R C T	220
601	GACCCTGAACGCGACATTTGCACGAAGACGTTCTACAAATACATTTCTCGTCGGTGCACG	660
221	Q P V T A I V S T L H Y N G K M R T T N	240
661	CAACCGGTGACAGCAATTGTGTCTACACTGCACTATAACGGAAAAATGCGCACCACCAAC	720
241	P C N K N I V I D I T G Q T K P K P G D	260
721	CCATGTAACAAGAACATCGTAATCGACATTACCGGACAAACCAACCAAAACCAGGAGAT	780
261	I I L T C F R G W V K Q L Q I E Y P G H	280
781	ATTATCCTGACGTGTTTCAGGGGGTGGGTCAAGCAGCTGCAGATTGAATACCCAGGACAC	840
281	E V M T A A V S G G L T R K G V F P V R	300
841	GAAGTTATGACTGCGGCAGTTTCAACAAGGATTGACGCGAAAAGGGTCTTTCCCGTAAGA	900
301	G K V N E N P L Y A I T S E H V N V L L	320
901	GGAAAAGTCAACGAGAACCCTTATATGCCATCACTTCTGAGCACGTCAACGTACTGTTG	960
321	T R T E D R I V W K T L Q G D P W I K Q	340
961	ACACGAACCGAAGATCGTATCGTGTGAAAACGCTACAAGGAGACCCTTGGATAAAGCAG	1020
341	L T N I P K G N F H A T V E E W E A E H	360
1021	CTCACAAACATTCCAAAAGGCAACTTTCACGCCACCGTCGAAGAATGGGAGGCTGAACAC	1080

Figure 8a. See legend on next page.

301	K G I M E A I I S F M F R K S N F F S L K	380
1081	AAGGGAATAATGGAGGCTATCACTAGCCCGGCCCGCCAGCAACCCTTTCAGCTGTAAG	1140
351	T N V C W A K A L E P I L S T A G I S L	400
1141	ACAAACGTGTGCTGGGCGAAGGCACTAGAACCTATACTATCGACCGCTGGCATATCACTA	1200
401	T G C Q W A D L F P Q F E D D K P H S A	420
1201	ACTGGATGTCAGTGGGCAGATTTGTTTCCGCAATTTGAAGATGACAAACCACATTTCGGCC	1260
421	I Y A L D V I C V K F F G M D L T S G I	440
1261	ATATACGCTCTAGACGTCATTTGCGTAAAGTTCTTTGGCATGGATTTAAGTAGCGGCATA	1320
441	F S K P L I P L T Y H P A E G D R K T A	460
1321	TTTTCAAACCGTTGATCCCATTTGACTTATCACCCCGCCGAAGGGGACCGGAAGACAGCG	1380
461	H W D N S P G Q R K Y G F D K A V V A E	480
1381	CACTGGGACAACAGTCCAGGCCAACGAAAGTACGGGTTTGACAAAGCCGTTGTAGCTGAA	1440
481	L S R R F P V F C M A D K G V Q L D L Q	500
1441	TTGTCCCGCAGATTCCCAGTATTCTGCGATGGCAGACAAAGGAGTGCAACTGGACCTACAG	1500
501	T G R T R V V ? S R F N L V P F N R N L	520
1501	ACGGGCCGNACGCGCGTAGTCNCGTCACGCTTCAACCTTGTGCCATTTAACAGAAATCTG	1560
521	P H S L V P E Y K T Q T P G Q L S A F I	540
1561	CCCCACTCGCTTGTCCCGGAGTATAAAACACAAACTCCAGGTCAGCTAAGCGCCTTTATC	1620
541	R Q F K Q N T I L L V S E T P A E H S T	560
1621	CGCCAGTTTAAACAAAACACCATCCTGCTTGTATCTGAAACACCTGCCGAACATTCCACC	1680
561	K S V E W I A P L G T L G A T K C Y N L	580
1681	AAATCTGTGGAATGGATTGCACCGCTGGGTACGCTTGGAGCCACCAATGCTATAATTTA	1740
581	A F G F P P Q S R Y D L V I I N I G T K	600
1741	GCATTTCGGCTTTCCGCCTCAGTCGAGGTACGACCTAGTGATCATAAATATCGGTACAAAA	1800
601	F R H H H Y Q Q C E D H A A T M K T L S	620
1801	TTCAGACACCACCACTATCAACAGTGCGAAGACCACGCCGCCACCATGAAGACACTGTCA	1860
621	R S A L N C L N P G G T L V V K A Y G Y	640
1861	CGTTCCGCCCTTAATTGCCTGAACCCGGGTGGCACATTGGTGGTAAAAGCATATGGCTAC	1920
641	A D R N S E D I I T A L A R K F V R V S	660
1921	GCGGACAGAAACAGTGAAGACATCATTACAGCCCTGGCAGCAAAGTTCGTCAGGGTGTCC	1980
661	A A R P Q C V S S N T E M Y F I F R Q L	680
1981	GCGGCCCGCCACAGTGGCTCTCAAGCAATACAGAGATGTACTTCATTTTCAGACAACTG	2040
681	D N S R T R Q F T P H H L N C V V S S V	700
2041	GACAACAGCAGAACACGTCAATTCACACCTCATCACCTCAACTGCGTCGTTTCGTCAGTG	2100
701	Y E G T R D G V G A	710
2101	TACGAGGGAACAAGAGACGGAGTTGGTGCT	2130

Figure 8b. Translated nucleotide sequence of Whataroa virus in the region encoding nonstructural protein nsP2. By homology with Sindbis virus, the sequence shown begins at amino acid 97 of nsP2 and continues to the nsP2/nsP3 cleavage site.

```

FINRKLYHIAVHGPAKNTEEEQYKAMRAEAADTEYVFDVDK K K CVKREEA
.V.....M.....VTK..L..E.....R...K...
      *           *           *           *           *
SGLVLVGELTNPPYHEMALEGLKTRPAVPYKVETIGVIGTPGSGKSAIIK
.....S.....L.....
      *           *           *           *           *
NIVTTRDLVTSGKKENCREIEADVLKHRKMQIVSKTVDSVLLNGCHKSV
ST..A.....RL..G...T.....M.....A..E
      *           *           *           *           *
ILYVDEAYACHAGTLLALIAIVRPRNKVVLCGDPKQCGFFNMMQLKVHFN
V.....F.....A.....K.....M.....
      *           *           *           *           *
DPERDICTKTFYKYISRRCTQPVTAIIVSTLHYNGKMRTTNPCNKNIVIDI
H..K.....D...K...K...E...
      *           *           *           *           *
TGQTKPKPGDIILTCFRGWVKQLQIEYPGHEVMTAAVSQGLTRKGVFPVR
..A.....D.....A.....YA...
      *           *           *           *           *
GKVNENPLYAITSEHVNVLLTRTEDRIVWKTLCQDPWIKQLTNIPKGNFH
Q.....L.....P.....Q
      *           *           *           *           *
ATVEEWEAEHKGIMEAITSAPRSNPFSCKTNVCWAKALEPILSTAGISL
..I.D.....IA..N..T..A.....A...V..
      *           *           *           *           *
TGCQWADLFPQFEDDKPHSAIYALDVICVKFFGMDLTSGIFSKPLIPTY
.....SE...A.....I.....L...QS....
      *           *           *           *           *
HPAEGDRKTAHWDNSPGQRKYGFDKAVVAELSRRFPVFCMADKGVQLDLG
...DSA.PV...T...Y.H.IA.....QL.G..T....
      *           *           *           *           *
TGRTRVV?SRFNLVPFNRNLPHSLVPEYKTQTPGQLSAFIQFKONTILL
.....ISAGH...V.....A.....EKQ..PVKK.LN...HHSV.V
      *           *           *           *           *
VSETPAEHSTKSVEWIAPLGTLGATKCYNLAFGFPPQSRDYDLVIINIGTK
...EKI.APR.RI....I..IA..D.N.....A.....F.....
      *           *           *           *           *
FRHHHYQGCEDHAATMKTLRSALNCLNPGGTLVVKAYGYADRNSEDIIT
Y.N..F.....L.....S.....VV..
      *           *           *           *           *
ALARKFVRVSAARPQCVSSNTEMYFIFRQLDNSRTRQFTPHHLNCCVSSV
.....D.....L.....I...
      *           *           *           *           *
YEGTRDGVGA
.....

```

Figure 9. Aligned deduced amino acid sequences of the nonstructural protein regions of Whataroa virus and Sindbis virus, beginning with amino acid 97 of Sindbis virus nsP2. The upper sequence in each case is Whataroa virus, and amino acid identity in the Sindbis sequence is indicated with a dot.

1	GCUCCGGCCUAUCGCUCGAAACGUGAGAAACAUCGCCGAGUGCCUCGAAGAGGCCGUAGUU	60
	A P A Y R S K R E N I A E C L E E A V V	
61	AAUGCCGCGAAUGCACUCGGACGGCCGGGCGAAGGGGUAUGCAAAGCCAUUAUAAAAAA	120
	N A A N A L G R P G E G V C K A I Y K K	
121	UGGCCUAAUAGUUUCGUCGAUUCGCGACAGAGACUGGAACGGCUAAGCUAGUGUGCUGU	180
	W P N S F V D S A T E T G T A K L V C C	
181	CAAGGAAAGAAAUAUCCACGCCGUCGGACCCGACUUCGCAAACACUCCGAGGCAGAA	240
	Q G K K I I H A V G P D F R K H S E A E	
241	GCACUGAAGAUUCUCCAGAACACAUACCAAGCCAUAGCAGAUUUGGUUAAACAAACAUUGA	300
	A L K I L Q N T Y H A I A D L V N K H G	
301	AUCAAGACUGUAGCGAUCCCGCUACUACCCACGGGAUUUACGCAGCGGGAAAAGACAGA	360
	I K T V A I P L L S T G I Y A A G K D R	
361	CUCGAGGUCUCCUAAACUGUCUUAACACCGCCUGGACAGAACAGACGCAGACGUCACA	420
	L E V S L N C L T T A L D R T D A D V T	
421	AUCUACUGUCUAGACAAAAAUGGAAAGAAAGGAUCGAUGCGGUUAUACAAUUGAAGGAG	480
	I Y C L D K K W K E R I D A V I Q L K E	
481	UCGGUGACGGAACUGAAGGAUGAGGAUAUGGAGAUUCGACGAUGAGUUAGUAUGGAUCCAC	540
	S V T E L K D E D M E I D D E L V W I H	
541	CCGGAUAGUUGUCUAAAGGGCAGGAAAGGGUAUAGCACAACAAAGGUAAACUUUAUUCG	600
	P D S C L K G R K G Y S T T K G K L Y S	
601	UACUUUGAGGGGACUAAGUUUCAUCAGGCAGCAAAGACAUGGCGGAGAUUAAAGUACUU	660
	Y F E G T K F H Q A A K D M A E I K V L	
661	UUUCCCGAUGAGCAAGAGUGCAACGAGCAGUUGUGUGCAUACAUCUUGGUGAAACCAUG	720
	F P D E Q E C N E Q L C A Y I L G E T M	
721	GAAGCCAUCAGGGAAAAAUGUCCAGUGGACUUUAAUCCGUCGUCCAGUCCGCCGAAGACA	780
	E A I R E K C P V D F N P S S S P P K T	
781	CUCCCCUGUUUGUGCAUGUAUGCCAUGACGCCUGAGAGAGUGCACCGUCUGCGUAGCAAC	840
	L P C L C M Y A M T P E R V H R L R S N	
841	AACGUCAAGUCCAUCACAGUGUGUUCGUCUACCCACUUCGGAAGCACAAGAUCAAGAAC	900
	N V K S I T V C S S T P L P K H K I K N	
901	GUUCAGAAAGUACAGUGCACGAAAGUGGUCUUGUUAUCCACAGACCCUGAAUUUGUC	960
	V Q K V Q C T K V V L F N P Q T P E F V	
961	CCUGCCCGUAAGUACAUAGAAGCACAACCAAAAGACGUAAGCCAAGAUGCAGAAGAAAGC	1020
	P A R K Y I E A Q P K D V S Q D A E E S	
1021	CCUGCCGAGCCGCCGAGAUAAACACCUCACGGGACGUAACAGACAUAUCCUGGAUGUG	1080
	P A A A A R D N T S R D V T D I S L D V	
1081	GAAGAAAGUCAAGCCGAGCCGGCCAACCAGAGGAGCGCUCGGGGGACAACACUCCCGG	1140
	E E S Q A A A G Q P E E R S G D N T S R	
1141	GAUGUAACAGAUUAUACCCUAGAUCACGACAGCGAUAGUGAGGUGGGUCUACUUCUCU	1200
	D V T D I S L D H D S D S E V G S I F S	
1201	AACCUAGCUGCUCCAGUCAAUCCAUCACUAGUAUGGACAGCUGGUCCUCCGGACCGGGA	1260
	N L S C S S Q S I T S M D S W S S G P G	

Figure 10a. See legend at the end of this sequence.

1261 UCGAUCACGAUAAACGAGAACCGCACCAUUCAGGUCACGGCGGAGAUACACAAUGCUCU 1320
 S I T I N E N R T I Q V T A E I H N A P
 1321 GCCGCGUUGCCUGUUCACACACGCCUUAAGAAACUGGCACGCUUAGCAGCCCAGAAG 1380
 A A L P V P P P R L K K L A R L A A Q K
 1381 CCCAAUCCGCCAUCCGACCCGCCUUCGACGGUCGAGGACGUGUCGAUGCGCUUGUCCUUC 1440
 P N P P S D P P S T V E D V S M R L S F
 1441 CCUGCCACGGUGUCGUUCGGAUCAUUCUCCGACGGAGAAGUCGACGACCUUAGCCGCGAU 1500
 P A T V S F G S F S D G E V D D L S R D
 1501 AAAGCAGUGUCAGAACCGGUGGUCUUGGUGCUUUCGAGCCUGGAGAGGUAACCUUAUC 1560
 K A V S E P V V F G A F E P G E V T S I
 1561 AUCGAAUCAAGGUCUGUCGUGUCAUCCCCGUGCAUAAACGCCGGCGCAGAAGACGGGGC 1620
 I E S R S V V S F P V H K R R R R R R G
 1621 AAAAGAACCGAUAUUGACUAACCGGGUAGGUGGGUACAUCUUCUCAACUGACACGGGA 1680
 K R T E Y * L T G V G G Y I F S T D T G
 1681 CCGGGCCACCUCAGAGAAGUCAGUUCUGCAAAACCAGCUUACUGAACCGACCCUCGAG 1740
 P G H L Q K K S V L Q N Q L T E P T L E
 1741 CGCAAUCAAUUAGAACGAAUGUAUGCGCCAGUCUGCAUGUCAAGAAAGAGGAACUUCUG 1800
 R N Q L E R M Y A P S L D V K K E E L L
 1801 AAACUUAAGUACCAAAUGAUGCCACCGAAGCCAAUAAAAGUAGGUACCAGUCUAGAAAG 1860
 K L K Y Q M M P T E A N K S R Y Q S R K
 1861 GUUGAAAAUCAAAAAGCGGUAACCACCGAGAGGUUACUGUCGGGACUGAAGAUGUACAUC 1920
 V E N Q K A V T T E R L L S G L K M Y I
 1921 CACUCAGAGAACCAACCUGAGUGUUAUAGGUCACUUAUCCGAAACCGUCGUACUCCAGC 1980
 H S E N Q P E C Y K V T Y P K P S Y S S
 1981 AGUGUCCCUUAGUUAACAGAACCCUGAAUUCGCCGUAGCUGUUGCAAUAACUACCUG 2040
 S V P L S Y Q N P E F A V A V C N N Y L
 2041 CAUGAGAACUACCCGACGGUUGCCUCCUAUCAGAUUACGGACGAAUAUGAUGCCUACCUC 2100
 H E N Y P T V A S Y Q I T D E Y D A Y L
 2101 GACAUGGUGGACGGCACUGUUGCGUGUCGACACUGCAACAUCUGCCCUGCGAAAUUA 2160
 D M V D G T V A C L D T A T F C P A K L
 2161 CGUAGCUUUCGAAAGAAACAUGAGUACCGCGCACCUAACAUCAGGAGUGCCGUGCCGUCU 2220
 R S F P K K H E Y R A P N I R S A V P S
 2221 GCUAUGCAGAACACUCUACAGAACGUCCUGAAUGCAGCAACAAGAGGAAUUGCAACGUU 2280
 A M Q N T L Q N V L N A A T K R N C N V
 2281 ACUCAGAUAGAGAAACUACCGACCCUAGACUCCGCGACCUUUAACGUGGAAUGCUUCCGA 2340
 T Q M R E L P T L D S A T F N V E C F R
 2341 AAGUACGCGUGCAAUGACGAGUAUUGGGUGAAUUCUCCGAAAAACCAUCAGGAUCACC 2400
 K Y A C N D E Y W A E F S E K P I R I T
 2401 ACGGAGUUUGUACGGCGUACGUGGCGAGAUGAAGGGACCAAAGGCUGCUGCUCUGUUU 2460
 T E F V T A Y V A R L K G P K A A A L F
 2461 GCAAAAACGCAUAACCUAGUCCCAUUGCAAGAAGUACCUAUGGACAGGUUUGUGAUGGAC 2520
 A K T H N L V P L Q E V P M D R F V M D

Figure 10b. See legend at the end of this sequence.

2521	AUGAAGCGAGAUGUCAAGGUGACUCCGGGCACAAAACACACCGAAGAAAGGCCUAAGGUG	2580
	M K R D V K V T P G T K H T E E R P K V	
2581	CAGGUAAUCCAAGCGGCUGAGCCUUUUGCUACAGCCUACCUUUGUGGCAUCCACCGAGAG	2640
	Q V I Q A A E P F A T A Y L C G I H R E	
2641	CUGGUACGCCGGCUUACCGCGGUUCUACUCCGAACGUACACACCCUGUUUGACAUGUCU	2700
	L V R R L T A V L L P N V H T L F D M S	
2701	GCGGAGGAUUCGACGCGAUUAUUGCCGAGCAUUCGACAAGGUGACGCCGUGCUCGAG	2760
	A E D F D A I I A E H F R Q G D A V L E	
2761	ACAGACAUCGCGUCAUUCGAUAAGAGUCAGGACGAUGCGAUGGCCUGACUGGGCUGAUG	2820
	T D I A S F D K S Q D D A M A L T G L M	
2821	AUCCUGGAGGACCUCGGCGUCGAUCAACCGCUGCUGGACCUCAUCGAGUGGCCUUCGGA	2880
	I L E D L G V D Q P L L D L I E C A F G	
2881	GAAUAUCAUCUACGCAUCUGCCUACUGGGACACGGUUUAAGUUCGGCUCAAUGAUGAAA	2940
	E I S S T H L P T G T R F K F G S M M K	
2941	UCCGGAUGUUCUACGCUCUUCGUGAACACCAUCUUGAAUGUCGUGAUCGCUAGUCGC	3000
	S G M F L T L F V N T I L N V V I A S R	
3001	GUGCUUGAGCACAGGUUAACAGGAUCACGAUGUGCCGCAUUCAUUGGAGACGAUAACAUC	3060
	V L E H R L T G S R C A A F I G D D N I	
3061	AUCCACGGCGUGGUUAUCAGACAAGGAAUUGGCCGAAAGGUGCGCCACUUGGCUGAAUUG	3120
	I H G V V S D K E M A E R C A T W L N M	
3121	GAGGUAAAAUCAUUGACGCGGUGAUCGGCGAGCGUCCUCCGUUUUCUGUGGUGGCUUU	3180
	E V K I I D A V I G E R P P Y F C G G F	
3181	AUACUACAGGACUCUGUCACCCAAACAGCCUGUCGAGUGGCUGACCCCCUAAAAAGACUG	3240
	I L Q D S V T Q T A C R V A D P L K R L	
3241	UUCAAGCUAGGAAAACCUUUGCCCGCAGAUGAUGACCAAGAUGAAGACAGAAGAAGGGCU	3300
	F K L G K P L P A D D D Q D E D R R R A	
3301	UUGCUGGAUGAGACUAAGGCGUGGUUAGAGUGGGCAUAACCGAAACAUUGGCUACUGCG	3360
	L L D E T K A W F R V G I T E T L A T A	
3361	GUAGCAACGCGGUACGAAGUUGAUAAUCAUCACGCCUGUCUGCUGGCACUGAGGACCCUU	3420
	V A T R Y E V D N I T P V L L A L R T L	
3421	GCGCAAAGCAAGAGAUCCUUCAGUCCAUAAGAGGGGAAUGAAGCAUCUCUACGGUGGU	3480
	A Q S K R S F Q S I R G E M K H L Y G G	
3481	CCUAAAUAG 3489	
	P K *	

Figure 10c. Nucleotide sequence of the region of the genome encoding nonstructural proteins nsP3 and nsP4 of Sindbis A1036, isolated in India in 1953. The sequence has been translated using the single letter amino acid code.

1	GCUCCGGCCUACCGCUCGAAACGUGAGAAUAUCGCCGAAUGCCUUGAAGAGGCCGUAGUU	60
	A P A Y R S K R E N I A E C L E E A V V	
61	AACGCCGCGAACCCACUCGGACGUCCGGGCGAAGGGGUGUGUAAAGCCAUAUAUAAAAAA	120
	N A A N P L G R P G E G V C K A I Y K K	
121	UGGCCCAAUAGUUUUGUGCAUUCUGCGACAGAGACUGGAACAGCUAAGCUAGUGUGCUGU	180
	W P N S F V D S A T E T G T A K L V C C	
181	CAAGGAAAAAGAUUAUCCAUGCCGUCGGACCUGACUCCGUAAACACCCCGAGGCAGAA	240
	Q G K K I I H A V G P D F R K H P E A E	
241	GCGCUGAAGAUUCCAGAAACACAUACCACGCCAUCGCAGAUUUGGUUAACAAACAUGGA	300
	A L K I L Q N T Y H A I A D L V N K H G	
301	AUCAAGACCGUAGCGAUCCCGCUUCUAUCCACCGGGAUUAACGCAGCGGGAAAAGACAGA	360
	I K T V A I P L L S T G I Y A A G K D R	
361	CUUGAGGUCUCUUAACUGCCUCACUACCGCCUGGACAGAACUGACGCAGACGUCACA	420
	L E V S L N C L T T A L D R T D A D V T	
421	AUCUACUGCCUUGACAAAAAUGGAAAGAACCGAUUGAUGCGUUUAUACAGUUGAAGGAG	480
	I Y C L D K K W K E R I D A F I Q L K E	
481	UCGGUGACGGAAACUGAAGGAUGAUGACAUGGAGAUCCGACGACGAAUAGUAUGGAUCCAC	540
	S V T E L K D D D M E I D D E L V W I H	
541	CCGGAUAGUUGCCUCAAGGGUAGGAAAGGGUUUAGUACGACGAAGGGCAAGCUCUACUCG	600
	P D S C L K G R K G F S T T K G K L Y S	
601	UACUUUGAGGGGACUAAAUUUAUCAAGCAGCAAAGACAUGGCUGAGAUCAGGUACUU	660
	Y F E G T K F H Q A A K D M A E I K V L	
661	UUUCCCGAUGAGCAAGAGUGCAACGAGCAACUGUGUGCAUACAUCUAGGCGAAACCAUG	720
	F P D E Q E C N E Q L C A Y I L G E T M	
721	GAAGCCAUCAGGGAAAAAUGUCCAGUGGACUUUAAUCCGUCGUCCAGUCCGCCGAAGACG	780
	E A I R E K C P V D F N P S S S P P K T	
781	CUUCCUGUUUGUGUAUGUACGCCAUGACGCCGAGAGAGUGCACCUCUUGCGUAGCAAU	840
	L P C L C M Y A M T P E R V H R L R S N	
841	AACGUCAAAUCCAUCACAGUAUGCUCGUCAACCCCGCUUCCGAAGCACAAAUAAGAAC	900
	N V K S I T V C S S T P L P K H K I K N	
901	GUUCAGAAAGUACAGUGCACGAAAGUAGUCCUAUUAACCCACAAACGCCUGAAUUGUC	960
	V Q K V Q C T K V V L F N P Q T P E F V	
961	CCUGCCCGCAAGUACAUAGAAACACAACCGAAGGACGACAGUCAAGAGGCGGAAGAAAAC	1020
	P A R K Y I E T Q P K D D S Q E A E E N	
1021	CCUGCCCGCAGCCGAUAACACUUCACGGGAUGUAACAGACGUAUCUCUAGAUUGUGGAAGGA	1080
	P A A A D N T S R D V T D V S L D V E G	
1081	GAUCGCGUUGCGGCCAACCGAUCAGAGGUGCACUCAGAGGACAACACCUCCCGAGAUGUA	1140
	D R V A A N R S E V H S E D N T S R D V	
1141	ACAGACAUUUCUAGACCACAACAGUGAUAGCGAGGUGGGCUCCAUUUUCUCUGACCUC	1200
	T D I S L D H N S D S E V G S I F S D L	
1201	AGCUGCUCCAGUCAUCCAUCACCAGCAUGGACAGCUGGUCCUCCGGACCGAGCUCGAUC	1260
	S C S S H S I T S M D S W S S G P S S I	

Figure 11a. See legend on the last page of this sequence.

1261	AUGCUAAACGGGAUACACACCAUCCAGGUCACGGCAGAGAUACACAACGCUCCUGCUGCA	1320
	M L N G N H T I Q V T A E I H N A P A A	
1321	CCGCCCCGUACACCACCACGCCUCAAGAAACUGGCGCGCUUGGCAGCUCAGAAGUCCGAU	1380
	P P V P P P R L K K L A R L A A Q K S D	
1381	CCGCCAUCCAGCCCCGCCCUAACGGUUGAGGACGUGUCGAUGCGCCUGUCAUUCCUGCC	1440
	P P S S P P S T V E D V S M R L S F P A	
1441	ACGGUGUCAUUCGGAUCUUUUUCUGACGGCGAAGUCGACGAUCUAGUCGCGAAAAAGCA	1500
	T V S F G S F S D G E V D D L S R E K A	
1501	GUGUCAGAACAGUGGUCUUUGGUGCUUUCGAGCCAGGAGAGGUAACAUCUAUCAUUGAA	1560
	V S E P V V F G A F E P G E V T S I I E	
1561	GCAAGGUCUGUCGUGUCAUUCGCCGUGAAUAAACGCCGGCGCAGGAGACGGGGCCAAAAG	1620
	A R S V V S F P V N K R R R R R R R G Q K	
1621	AAAACCGAAUAUUGACUAACCGGGUAGGUGGGUUAUCUUCUGACUGACACGGGACCG	1680
	K T E Y * L T G V G G Y I F S T D T G P	
1681	GGUCACCUCCAGAAAAAUCCGUUCUACAAACAGCUUACGGAACCGACCCUCGAGCGU	1740
	G H L Q K K S V L Q N Q L T E P T L E R	
1741	AAUCAAUUAGAACGAGUGUAUGCACCCAGUCUUGAUGCCAGAAAGAGGAAACUCUUGAAA	1800
	N Q L E R V Y A P S L D A K K E E L L K	
1801	CUCAAGUACCAAUGAUGCCACCGAAGCCAAUAAAAGUAGGUACCAGUCUAGAAAGGUA	1860
	L K Y Q M M P T E A N K S R Y Q S R K V	
1861	GAAAACCAAAGCCGUAACACCGAGAGGUUACUGUCGGGAUUGAAGAUACAUCAC	1920
	E N Q K A V T T E R L L S G L K M Y I H	
1921	UCAGAGAACCAACCCGAGUGUUAACAAGGUACCUAUCCGAAACCGUCGUACUCUAGCAGU	1980
	S E N Q P E C Y K V T Y P K P S Y S S S	
1981	GUUCCCCUAGUUAACAGAGCCCCGAAUUCGCCGUAGCCGUCUGCAAUAACUACCUGCAU	2040
	V P L S Y Q S P E F A V A V C N N Y L H	
2041	GAGAAUUAUCCAACGGUUGCCUCCUAUCAGAUUACGGAUGAAUAUGACGCCUACCUUGAC	2100
	E N Y P T V A S Y Q I T D E Y D A Y L D	
2101	AUGGUGGACGGCACCGUAGCGUGUCUGACACCGCUACAUUUUGCCCCGCGAAAUUACGC	2160
	M V D G T V A C L D T A T F C P A K L R	
2161	AGCUUCCCCAAGAAACACGAGUACCGAGAACCUAACAUCAGGAGCGCCGUACCGUCCGCU	2220
	S F P K K H E Y R E P N I R S A V P S A	
2221	AUGCAGAACACUCUACAGAACGUCCUGAACGCAGCAACAAAGAGGAAUUGCAAUGUUAUCU	2280
	M Q N T L Q N V L N A A T K R N C N V T	
2281	CAGAUGAGAGAACUACCGACUUUAGACUCGCAACCUUUAAUGUGGAAUGCUUUCGAAAG	2340
	Q M R E L P T L D S A T F N V E C F R K	
2341	UACGCGUGCAACGACGAGUAUUGGGCUGAAUUCUCCGAAAAACCAAUUAGGAUCACCACA	2400
	Y A C N D E Y W A E F S E K P I R I T T	
2401	GAGUUUGUCACGGCGUACGUGGCGAGAUUGAAGGGACCAAAGGCUGCUGCACUGUUUGCU	2460
	E F V T A Y V A R L K G P K A A A L F A	
2461	AAAACGCAUAACCUAGUCCACUGCAAGAAGUACCUAUGGACAGGUUUGUGAUGGACAUG	2520
	K T H N L V P L Q E V P M D R F V M D M	

Figure 11b. See legend on the last page of this sequence.

2521 AAGCGAGACGUUAAGGUGACUCCGGGCACGAAGCACACCCGAAGAAAGACCCAAAGUGCAG 2580
 K R D V K V T P G T K H T E E R P K V Q
 2581 GUAAUCCAAGCGGCAGAGCCUCUAGCUACAGCCUAUUUAUGCGGCAUCCACCGUGAGCUG 2640
 V I Q A A E P L A T A Y L C G I H R E L
 2641 GUACGCAGGCUUACCGCAGUCCUGCUUCCGAACGUACACACCCUUUUUGAUUGUCUGCG 2700
 V R R L T A V L L P N V H T L F D M S A
 2701 GAAGAUUUCGAUGCUAUAUUGCCGAGCAUUUUCACCAGGGUGACGCUGUGCUCGAGACA 2760
 E D F D A I I A E H F H Q G D A V L E T
 2761 GACAUCGCGUGCUUGGAUAAGAGCCAAGACGAUGCGAUGGCCUGACGGGCGUGAUGAUC 2820
 D I A S F D K S Q D D A M A L T G L M I
 2821 CUGGAGGACCUCGGAGUCGACCAGCCAUUGCUGGACCUCUAGCAGUGCGCCUUCGGGGAA 2880
 L F D L G V D Q P L L D L I E C A F G E
 2881 AUAUCAUCUACGCACCUGCCGACCGGGACACGGUUUAAGUUCGGCUCAAUGAUGAAAUCC 2940
 I S S T H L P T G T R F K F G S M M K S
 2941 GGAAUGUUCUCACGCUCUUUGUGAACACCAUCUUGAAUGUCGUGAUAGCUAGUCGCGUG 3000
 G M F L T L F V N T I L N V V I A S R V
 3001 CUCGAGCACAGGUUAGCAGAAUCACGAUGCGCCGCAUUCUAGCAGACGACAAUUAUUU 3060
 L E H R L A E S R C A A F I G D D N I I
 3061 CACGGCGUGGUUAUCCGACCAAGAAUUGGCUGAAAGGUGCGCCACUUGGCUGAAUAUGGAG 3120
 H G V V S L K E M A E R C A T W L N M E
 3121 GUAAAAAUUAUUCGAGUAAUUGGCGAACGUCCUCCGUACUUCUGUGGCGGCUUUUAU 3180
 V K I I D A V I G E R P P Y F C G G F I
 3181 CUGCAGGACUCAGUCACCCAAACAGCCUGCCGAGUGGCGGACCCCCUAAAAAGAUUGUUC 3240
 L Q D S V T Q T A C R V A D P L K R L F
 3241 AAAUUGGAAAACCAUUAUCCUGCAGAUGAUGACCAAGAUGAAGACAGAAGAAGGGGUCUG 3300
 K L G K P L P A D D D Q D E D R R R A L
 3301 CUGGAUGAGACCAAGGCGUGGUUAGAGUGGGCAUAACUGAGACACUGGCUCUGCGGUA 3360
 L D E T K A W F R V G I T E T L A T A V
 3361 GCAACGCGGUUGAAGUUGAUAAUCAACACCGGUCCUGCUGGCACUGAGGACCCUUGCG 3420
 A T R Y E V D N I T P V L L A L R T L A
 3421 CAAAGCAAGAGAUUUUCAGGCCAUAGGGGGAAAAUGAAGCAUCUCUACGGUGGUCCU 3480
 Q S K R S F Q A I R G K M K H L Y G G P
 3481 AAUAG 3486
 K *

Figure 11c. Nucleotide sequence of the region of the genome encoding nonstructural proteins nsP3 and nsP4 of an isolate of Sindbis virus isolated from a mosquito pool from Australia in 1975.

1 GCACCGUCAUACCGCACUAAAAGGGAGAACAUUGCUGAUUGUCAAGAGGAAGCAGUUGUC 60
 A P S Y R T K R E N I A D C Q E E A V V
 61 AAUGCAGCCAAUCCGCUGGGAGACCAGGCGAAGGAGUCUGCCGUGCCAUUCUAAAACGU 120
 N A A N P L G R P G E G V C R A I Y K R
 121 UGGCCGAACAGUUUACCCGAUUCAGCCACAGAGACCGGCACCGCAAACUGACUGUGUGC 180
 W P N S F T D S A T E T G T A K L T V C
 181 CAAGGAAAGAAAGUGAUCCACGCGGUUGGCCUGAUUUCGGAAACACCCAGAGGCAGAA 240
 Q G K K V I H A V G P D F R K H P E A E
 241 GCCCUGAAAUUGCUGCAAAACGCCUACCAUGCAGUGGCAGACUAGUAAAUGAACAUAAU 300
 A L K L L Q N A Y H A V A D L V N E H N
 301 AUCAAGUCUGUGCCAUCCACUGCUAUCUACAGGCAUUUACGCAGCCGGAAGACCGC 360
 I K S V A I P L L S T G I Y A A G K D R
 361 CUUGAAGUAUCACUUAACUGCUUGACAACCGCGCUAGAUAGAACUGAUGCGGACGUAACC 420
 L E V S L N C L T T A L D R T D A D V T
 421 AUCUACUGCCUGGAUAAGAAGUGGAAGGAAAGAAUCGACGCGGUGCUCCAACUUAAGGAG 480
 I Y C L D K K W K E R I D A V L Q L K E
 481 UCUGUAACAGAGCUGAAGGAUGAGGAUAUGGAGAUUCGACGACGAGUUAGUAUGGAUCCA 540
 S V T E L K D E D M E I D D E L V W I H
 541 CCGGACAGUUGCCUGAAGGGAAGAAAGGGAUUCAGUACUACAAAAGGAAAGUUGUAUUCG 600
 P D S C L K G R K G F S T T K G K L Y S
 601 UACUUUGAAGGCACCAAAUCCAUAAGCAGCAAAAGAUUUGGCGGAGAUAAAGGUCCUG 660
 Y F E G T K F H Q A A K D M A E I K V L
 661 UUCCCAAUUGACCAGGAAAGCAACGAGCAACUGUGUGCCUACAUUUGGGGGAGACCAUG 720
 F P N D Q E S N E Q L C A Y I L G E T M
 721 GAAGCAAUCCGCGAAAAUUGCCCGGUGGACCAACCCGUGUCUAGCCCGCCAAAAACG 780
 E A I R E K C P V D N P S S S P P K T
 781 CUGCCGUGCCUCUGCAUGUAUGCCAUGA. GGCAGAAAGGGUCCACAGACUCAGAAGCAAC 840
 L P C L C M Y A M T P E R V H R L R S N
 841 AACGUCAAAGAAGUACAGUAUGCUCCUCCACCCCCUCCAAAGUACAAAUCAAGAAC 900
 N V K E V T V C S S T P L P K Y K I K N
 901 GUUCAGAAGGUUCAGUGCACAAAAGUAGUCCUGUUUAACCCGCAUACCCUGCAUUCGU 960
 V Q K V Q C T K V V L F N P H T P A F V
 961 CCCGCCCGUAAGUACAUAGAAGCGCCAGAACAGCCUGCAGCUCCGCCUGCACAGGCCGAG 1020
 P A R K Y I E A P E Q P A A P P A Q A E
 1021 GAGGCCCCCGAAGUUGCAGCAACACCAACACCACUGCAGCUGAUAAACCCUCGCUUGAU 1080
 E A P E V A A T P T P P A A D N T S L D
 1081 GUCACGGACAUCUCACUGGACAUGGAAGACAGUAGCGAAGGCUCACUCUUUUCGAGCUUU 1140
 V T D I S L D M E D S S E G S L F S S F
 1141 AGCGGAUCGGACAACUCUAUUACUAGUAUGGACAGUUGGUGUCAGGACCUAGUUCACUA 1200
 S G S D N S I T S M D S W S S G P S S L
 1201 GAGAUAGUAGACCGAAGGCAGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGGUGG 1260
 E I V D R R Q V V V A D V H A V Q E P A

Figure 12a. See legend on last page of this sequence.

1261	CCUGUUCCACCGCCAAGGCUAAAGAAGAUGGCCCGCCUGGCAGCGGCAAGAAUGCAGGAA P V P P P R L K K M A R L A A A R M Q E	1320
1321	GAGCCAACUCCACCGCAAGCACCAGCUCUGCGGACGAGUCCCUUACCUCUUUUUGGU E P T P P A S T S S A D E S L H L S F G	1380
1381	GGGGUAUCCAUGUCCUUCGGAUCCUUUUUGACGGAGAGAUGGCCCGCUUGGCAGCGGCA G V S M S F G S L F D G E M A R L A A A	1440
1441	CAACCCCCGGCAAGUACAUGCCCUACGGAUGUGCCUAUGUCUUUCGGAUCGUUUUCCGAC Q P P A S T C P T D V P M S F G S F S D	1500
1501	GGAGAGAUUGAGGAGCUGAGCCCGCAGAGUAACCGAGUCUGAGCCCGUCCUGUUUGGGUCA G E I E E L S R R V T E S E P V L F G S	1560
1561	UUUGAACCGGGCGAAGUGAACUCAUUUAUAUCGUCCCCGAUCAGCCGUAUCUUUUCCACCA F E P G E V N S I I S S R S A V S F P P	1620
1621	CGCAAGCAGAGACGUAGACGCAGGAGCAGGAGGCCGAUACUGACUAACCGGGGUAGGU R K Q R R R R R S R R T E Y * L T G V G	1680
1681	GGGUACAUAUUUUCGACGGACACAGGCCUUGGGCACUUGCAAAGAAGUCCGUUCUGCAG G Y I F S T D T G P G H L Q K K S V L Q	1740
1741	AACCAGCUUACAGAACCGACCUUGGAGCGCAAUGUUCUGGAAAGAAUCUACGCCCCGGUG N Q L T E P T L E R N V L E R I Y A P V	1800
1801	CUCGACACGUCGAAAGAGGAACAGCUCAAACUCAGGUACCAGAUGGCCACCGAAGCC L D T S K E E Q L K L R Y Q M M P T E A	1860
1861	AACAAAAGCAGGUACCAGUCUAGAAAAGUAGAAAUCAGAAAGCCAUAAACACUGAGCGA N K S R Y Q S R K V E N Q K A I T T E R	1920
1921	CUGCUUUCAGGGCUACGACUGUAUAACUCUGCCACAGAUCAGCCAGAAUGCUAUAAGAUC L L S G L R L Y N S A T D Q P E C Y K I	1980
1981	ACCUACCCGAAACCAUCGUUUCAGCAGUGUACCGGCGAACUACUCUGACCCAAAGUUU T Y P K P S Y S S S V P A N Y S D P K F	2040
2041	GCUGUAGCUGUUUGCAACAACUAUCUGCAUGAGAAUACCCGACGGUAGCAUCUUAUCAG A V A V C N N Y L H E N Y P T V A S Y Q	2100
2101	AUCACCGACGAGUACGAUGCUUACUUGGAUAUGGUAGACGGGACAGUCGCUUGUCUAGA I T D E Y D A Y L D M V D G T V A C L D	2160
2161	ACUGCAACUUUUUGCCCCGCAAGCUUAGAAGUUACCCGAAAAGACACGAGUAUAGAGCC T A T F C P A K L R S Y P K R H E Y R A	2220
2221	CCAAACAUCGCGCAGUGCGGUUCCAUCAGCGAUGCAGAACACGUUGCAAACGUGCUCAUU P N I R S A V P S A M Q N T L Q N V L I	2280
2281	GCCGCGACUAAAAGAAACUGCAACGUCACACAAUGCGUGAAUUGCCAACACUGGACUCA A A T K R N C N V T Q M R E L P T L D S	2340
2341	GCGACAUUCAACGUUGAAUGCUUUCGAAAUAUGCAUGUAUGACGAGUAUUGGGAGGAG A T F N V E C F R K Y A C N D E Y W E E	2400
2401	UUUGCCCGAAAGCCAAUAGGAUCACUACUGAGUUCGUUACCGCAUACGUGGCCAGACUG F A R K P I R I T T E F V T A Y V A R L	2460
2461	AAAGGCCCUAAGGCCGCCGCACUGUUCGCAAGACGCAUAAUUGGUCCCAUUGCAAGAA K G P K A A A L F A K T H N L V P L Q E	2520

Figure 12b. See legend on the last page of this sequence.

2521 GUGCCUAUGGAUAGGUUCGUGCAUGGACAUGAAAAGAGACGUGAAAGUUACACCUGGCAGC 2580
 V P M D R F V M D M K R D V K V T P G T
 2581 AAACACACAGAAGAAAGACCGAAAGUACAAGUGAUACAGCCGCAGAACCCUGGCGACC 2640
 K H T E E R P K V Q V I Q A A E P L A T
 2641 GCUUACCUGUGCGGGAUCCACCGGGAGUAGUGCGCAGGCUUACAGCCGUCUUGCUACCC 2700
 A Y L C G I H R E L V R R L T A V L L P
 2701 AACAUUCACACGCUUUUUGACAUGUCGGCGGAGGACUUGAUGCAAUCAUAGCAGAACAC 2760
 N I H T L F D M S A E D F D A I I A E H
 2761 UUCAAGCAAGGUGACCCGGUACUGGAGACGGAUUCGCCUCGUUCGACAAAAGCCAAGAC 2820
 F K Q G D P V L E T D I A S F D K S Q D
 2821 GACGCUAUGGCGUUAACUGGCCUGAUGAUUUGGAAGACCUGGGUGUGGACCAACCACUA 2880
 D A M A L T G L M I L E D L G V D Q P L
 2881 CUCGACUUGAUCGAGUGCGCCUUUGGAGAAAUUCAUCCACCCAUCUGCCCACGGGUACC 2940
 L D L I E C A F G E I S S T H L P T G T
 2941 CGUUUCAAUUCGGGGCGAUGAUGAAAUCCGGAUUGUCCUCACGCUCUUGUCAACACA 3000
 R F K F G A M M K S G M F L T L F V N T
 3001 GUUCUGAAUGUCGUUAUCGCCAGCAGAGUAUUGGAGGAGCGGCUUAAAACGUCCAAUUG 3060
 V L N V V I A S R V L E E R L K T S K C
 3061 GCAGCAUUAUUCGGCGACGACAACAUAUACACGGAGUAGUAUCUGACAAAGAAUUGGU 3120
 A A F I G D D N I I H G V V S D K E M A
 3121 GAGAGGUGUGCCACCUGGCUCAACAUGGAGGUUAAGAUAUUGACGCAGUCAUCGGCGAG 3180
 E R C A T W L N M E V K I I D A V I G E
 3181 AGACCGCCUACUUCUGCGGUGGAUUAUCUUGCAAGAUUCGGUUAACCUCCACAGCGUG 3240
 R P P Y F C G G F I L Q D S V T S T A C
 3241 CGCGUGGCGGACCCCUUGAAAAGGCUGUUUAAGUUGGGUAAACCGCUCCAGCCGACGAC 3300
 R V A D P L K R L F K L G K P L P A D D
 3301 GAGCAAGACGAAGACAGAAGACGCGCUCUGCUAGAUGAAACAAAGGCGUGGUUAGAGUA 3360
 E Q D E D R R R A L L D E T K A W F R V
 3361 GGUAUAACAGACACCUUAGCAGUGGCCGUGGCAACUCGGUAUGAGGUAGACAACAUCACA 3420
 G I T D T L A V A V A T R Y E V D N I T
 3421 CCUGUCCUGCUGGCAUUGAGAACUUUGGCCAGAGCAAAAGAGCAUUUCAAGCCAUCAGA 3480
 P V L L A L R T F A Q S K R A F Q A I R
 3481 GGGGAAAUAAAGCAUCUCUACGGUGGUCCUAAAUAG 3516
 G E I K H L Y G G P K *

Figure 12c. Nucleotide sequence of the region of the genome encoding nonstructural proteins nsP3 and nsP4 for the Girdwood South African strain of Sindbis virus isolated in 1963.

viral disease. These viruses are all closely related, exhibiting 90% or greater amino acid sequence identity in the conserved region of nsP3 or in nsP4. Conclusions as to sequence relationships are similar to conclusions drawn from the analysis of the 3' NTR.

CONCLUSIONS

We have identified an important antigenic epitope present in E2 of the alphaviruses. This epitope, located in whole or in part within the domain of E2 between residues 170 and 220, depending upon the antibody, is clearly of major importance for the neutralization of the virus infectivity and thus for vaccine design.

We have established the relationships between many of the Sindbis-like alphaviruses. The Sindbis-like viruses, which are found throughout the Old World from Northern Europe to Africa, India, the Philippines and the Australasian region including New Guinea, are a clearly identifiable group of viruses. They share a minimum of 80% amino acid sequence identity in the nonstructural proteins and possess a characteristic and conserved 3' NTR. Virulent strains exist that can cause significant disease in man, and the relationship of the virulent strains to avirulent strains has been established. It is of considerable interest that viruses belonging to this group coexist in many parts of the world with other alphaviruses that are demonstrably different in their epidemiology, serology, organization of the 3' NTR, and evolutionary history, even though many of these non-Sindbis alphaviruses cause diseases very similar to those caused by the virulent Sindbis-like viruses.

We found that a strain of Sindbis virus from Northern Europe that causes Ockelbo disease in Sweden, Pogosta disease in Finland, or Karelian fever in

Russia, a disease characterized by a polyarthritits whose symptoms can persist for months or years, are very closely related to pathogenic strains of Sindbis virus isolated from South Africa. We concluded that a South African strain of Sindbis was introduced into Northern Europe, probably in the 1960s, where it continues to cause epidemics of a significant human disease (Shirako et al., 1991).

We have shown that Aura virus is a New World representative of the Sindbis viruses. Further analysis is required to determine whether it is one of the parents of Western equine encephalitis virus, but the hypothesis that Western equine encephalitis virus is a virus that emerged from a recombination event has received further support from these studies.

We have also shown that high throughput automated DNA sequencing is ideally suited to the rapid analysis of an RNA virus family such as the alphaviruses. These procedures are rapid and generate large amounts of useful information very quickly. Such procedures would be very useful in defining the origin and spread of an epidemic virus.

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